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Regulation of FSH receptor, PKIβ, IL-6 and calcium mobilization: Possible mediators of differential action of FSH

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Abstract

Sertoli cells support the development of germ cells by providing a microenvironment in the seminiferous tubules. FSH stimulates Sertoli cell proliferation only during neonatal period till day 18 in the immature rat whereas FSH regulates only functional parameters in the adult rat Sertoli cells. This suggests that FSH exerts differential action in immature and adult Sertoli cells. In an attempt to elucidate the mechanism by which FSH exerts the differential effects, we have carried out both in vivo and in vitro studies using Sertoli cells isolated from immature (7–10 days old) and adult (90 days old) rats. The differential role of FSH was studied at the receptor as well as at the signaling level. Monitoring the level of expression of FSH receptor by RTPCR and northern blot analysis revealed that the expression was more in immature Sertoli cells. Furthermore, it was found that FSH up (1.8-fold) regulates its receptor level only in the immature Sertoli cells and not in the adult. Results also revealed that PKIβ and calcium, which are the downstream signaling molecules, are involved in FSH regulated Sertoli cells proliferation. It was also observed that FSH up (1.4-fold) regulates the levels of expression of IL-6 mRNA only in the immature rat Sertoli cells suggesting the possibility of its involvement in FSH regulated Sertoli cell proliferation.

Keywords: Sertoli cells; FSH receptor; IL-6; Protein kinase inhibitor; Calcium

1. Introduction

Sertoli cells play a very crucial role in male reproduction by serving as the nurse cells for the spermatogonial cells which continue to divide, although the proliferation of Sertoli cells ceases shortly after birth in rodents. In rats, the Sertoli cell proliferation begins during the prenatal period and continues up to day 15–18 postnatal, but beyond this period the Sertoli cells do not divide (Orth, 1982). Follicle stimulating hormone (FSH) is the main mitogen for Sertoli cells (Orth, 1984; Kumar et al., 1997). During day 14–21, coincident with arrest of proliferation, Sertoli cells also undergo a differentiation process under the influence of FSH. Both morphological and functional changes which includes production of secretory proteins, like transferrin and androgen binding protein (ABP) needed by germ cells, as well as synthesis of other proteins that are involved in the formation of specialized tight junctions between neighboring Sertoli cells to establish the blood-testis barrier have been reported (Means et al., 1980). FSH also stimulates synthesis of a number of growth factors and interleukins in adult Sertoli cells which have both autocrine and paracrine role on the developing spermatogonia (Parvinen, 1982). Of the various growth factors known to be produced by Sertoli cells IL-1 and IL-6 are important and interestingly IL-1 is not regulated by FSH (Stephan et al., 1997). The exit of Sertoli cells from the cell cycle does not appear to be due to decrease in FSH as its levels raises steadily during early postnatal development in rodents.

Studies have revealed that following interaction of FSH with its cognate receptor, there is increase in cyclic AMP (cAMP) production (Kangasniemi et al., 1990), growth factors (Feig et al., 1980; Saez et al., 1987; Avallet et al., 1997) and calcium mobilization (Grasso and Reichert, 1989; Grasso et al., 1991). The differential action of FSH on Sertoli cells at the two different stages of development (immature and adult) can be due to a number of factors like the differential expression of receptors for FSH, differential regulation of downstream molecules or gene expression. Although considerable information is available on the actions of FSH on Sertoli cells, the possible reasons for the differential action of FSH in regulation of proliferation in

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immature Sertoli cell and functional parameters in adult Sertoli cells have not been investigated.

In the present study, we have attempted to analyze the possible mechanism by which FSH exerts differential action during the two stages of Sertoli cell development. The results of the study which involved monitoring the action of addition of FSH or deprival of FSH under in vitro and in vivo conditions on Sertoli cells from immature and adult rat are presented.

2. Materials and methods

2.1. Animals and treatment

Seven-day-old and 90-day-old male Wistar rats were otained from the Central Animal Facility, Indian Institute of Science, Bangalore, India and maintained under standard conditions (12 h of light and 12 h of dark schedule, with water and pelleted food ad libitum). The animal procedures employed in the study have been approved by Institutional Ethical Committee.

Antiserum to highly purified ovine FSH (oFSH) was raised in adult male bonnet monkey (*Maccaca radiata*). The absence of contaminating antibodies to LH was established by lack of binding to ^{125}I-hCG and the inability of the antiserum to inhibit the LH stimulated testosterone production by the Leydig cells. The effect of antiserum to neutralize endogenous FSH was established in immature male rats. Normal monkey serum (NMS) administered rats served as controls.

In order to study the effect of deprival of FSH on growth and function of Sertoli cells, 7-day-old immature rats and 90-day-old adult rats were administered 100 and 300 μl of FSH a/s or NMS by i.p. route twice a day for 5 days. On the sixth day the effect of neutralization of endogenous FSH was analyzed.

2.2. Isolation of Sertoli cell and culture

Sertoli cells were isolated from immature rats by previously described protocol (Tung and Fritz, 1998); briefly, seminiferous tubules were digested in 0.125% of Trypsin (Sigma Chemical Co., St. Louis, MO) and 10 μg/ ml of DNase (Worthington) in 1× HBSS for 20 min. After three washes in 1× HBSS the tubules were subjected to two enzymatic digestions with 1 mg/ml collagenase (Worthington) and DNase followed by 1 mg/ml collagenase, 1 mg/ml hyaluronidase (Sigma Chemical Co., St. Louis, MO) and DNase for 30 min each. The cells were washed thrice with 1× HBSS and pelleted down by centrifugation. The cells were resuspened in 1 ml of DMEM Ham-F12 (Sigma Chemical Co., St. Louis, MO) and counted in hemaetocytometer. Immature Sertoli cells were maintained in DMEM Ham-F12 medium containing 10 μg/ml insulin (Sigma Chemical Co., St. Louis, MO) and 5 μg/ml transferrin (Sigma Chemical Co., St. Louis, MO) at 32 °C in 5% CO_2. After 24 h of preculture, Sertoli cells were subjected to hypotonic shock by replacing the medium with medium:water::1:10. The purified Sertoli cells were incubated for 8 h in medium containing 250 ng/ml oFSH (oFSH was a kind gift from Dr. M. R. Sairam, IRCM, Montreal) or vehicle (medium only).

Sertoli cells were isolated from adult rats using a similar protocol employed for immature rats except with some modifications. Briefly, following the digestion of seminiferous tubules with trypsin, collagenase and hyaluronidase, the mixed population of cells was passed twice through glass wool to get rid of sperms. The cells were washed twice with 1× HBSS and pelleted down by centrifugation, resuspened in 1 ml of DMEM Ham-F12 and counted in hemaetocytometer. The culture and incubation conditions for adult Sertoli cells were exactly same as that employed for immature Sertoli cells. Viability of the immature and adult rat Sertoli cells at the end of experiments was analyzed by MTT assay (data not presented). The purity of the Sertoli cells was determined by assessing for the absence of germ cells as monitored under the phase contrast microscope (Fig. 1); for the absence of Leydig cells by checking for LH receptor (LH-R) by RTPCR (Fig. 2A and B) and for the absence of myoid cells by analyzing for the alkaline phosphatase staining (Fig. 2C and D).

2.3. Semi-quantitative RTPCR

Total RNA was extracted from cells or tissues using TRI reagent (Sigma Chemical Co., St. Louis, MO). To eliminate the genomic DNA contamination from the RNA, the RNA samples were treated with RNase-free DNase. Four micrograms RNA was treated with 1 μL of RQ1 RNase-free DNase in a 1× reaction mixture of RQ1 DNase buffer plus 1 μL of RNasin® (RNase Inhibitor from Amersham Pharmacia) for 30 min at 37 °C. The reaction was stopped by the addition of RQ1 DNase stop solution and incubating the mixture at 65 °C for 10 min. Following DNase (Amersham Pharmacia Biotech., UK) treatment, 2 μg of total RNA was subjected to reverse transcription using

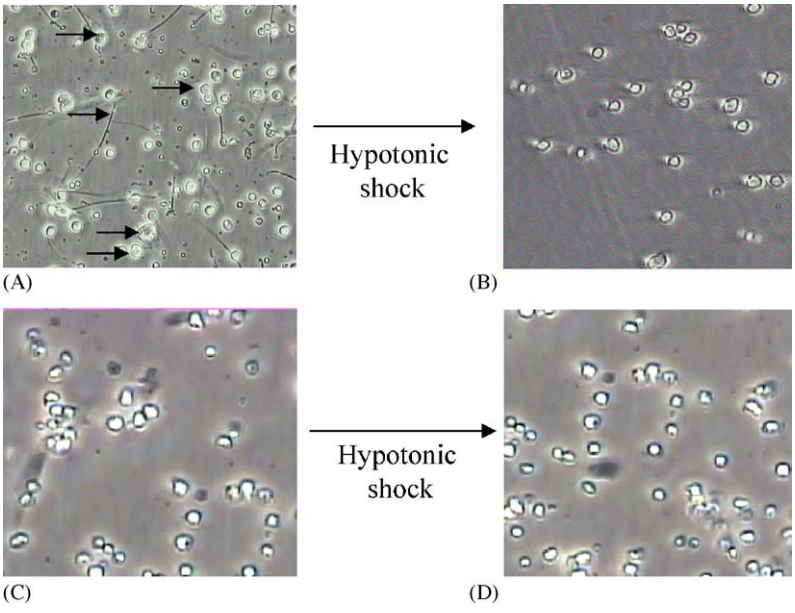

Fig. 1. Characterization of Sertoli cells for the absence of germ cells. Phase contrast microscopic images of Sertoli cells isolated from the adult rat testis before (A) and after (B) hypotonic shock. After the hypotonic shock a homogeneous population of cells was obtained (B). The Sertoli cells isolated from the immature rat testis revealed a homogeneous population of cells both before (C) as well as after (D) the hypotonic shock (original magnification 40×). The figures are representative of three independent experiments.